



ORAL PRESENTATION

Open Access

HCV full-length genome reconstruction with sequence independent amplification combined with next generation sequencing

Barbara Bartolini*, Emanuela Giombini, Isabella Abbate, Ubaldo Visco-Comandini, Gianpiero D'offizi, Gabriella Rozera, Marina Selleri, Giuseppe Ippolito, Maria Rosaria Capobianchi

From 17th International Symposium on HIV and Emerging Infectious Diseases (ISHEID)
Marseille, France. 23-25 May 2012

Introduction

HCV genome variability is related to both disease progression and treatment response. De novo high-throughput pyrosequencing was used to obtain full length HCV genome characterization directly from clinical samples.

Material and methods

Plasma samples from 3 HCV-infected subjects were analyzed (two patients with subtype 1b, one patient with subtype 2a/2c; viral load: 6.0×10^6 , 20.8×10^6 and 7.3×10^6 IU/ml viral load, respectively). All samples were analyzed in a single run, using sample-specific barcoding adapters. Data were generated with a modified sequence-independent single primer amplification followed by 454 sequencing (GS-FLX Roche, Titanium version), using the shotgun approach. The reads were assembled using cap3 program; HCV contigs were identified using BLAST against full HCV genome database. Reads of HCV contigs were used for genome reconstruction with gs Mapper (Roche software).

Results

A total of 297,493 reads were obtained (average length 267 bp). Using a minimum read length cut off of 40 nt with >90% identity and >40% overlapping, BLAST analysis classified a total of 27,107 reads (10,682 from patient 1, 11,920 from patient 2, and 4,505 from patient 3) as HCV-specific. In all patients, genome reconstruction was achieved for more than 98 % of the entire HCV genome. The mean coverage was 315, 307 and 142 reads per site for patients 1, 2 and 3, respectively (overall mean coverage: 253 reads

per site). Within-patient variability was calculated, resulting in E1 and E2 as the most variable structural genes in all patients, as expected.

Conclusion

The present study describes a unifying approach for HCV full genome sequencing, based on sequence-independent amplification combined with next generation sequencing. This may represent a relevant innovation, since so-far HCV full genome direct sequencing was based on genotype-specific multiple primer approach and conventional sequencing. The possibility of performing simultaneous analysis of pooled samples may represent a further advantage for cost saving. High coverage allows to analyze virus variability along the entire genome providing important information on possible viral variants which could impact on clinical and therapeutic outcome.

Published: 25 May 2012

doi:10.1186/1742-4690-9-S1-O6

Cite this article as: Bartolini et al.: HCV full-length genome reconstruction with sequence independent amplification combined with next generation sequencing. *Retrovirology* 2012 **9**(Suppl 1):O6.

* Correspondence: barbara.bartolini@inmi.it

Virology at National Institute for Infectious Diseases L. Spallanzani, Rome, Italy